Comparison of Different Methods for the Determination of the Oil Content in Oilseeds

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ABSTRACT: The investigation and assessment of the oil content of oilseeds are important criteria, especially for the oil milling trade. Standard methods for the determination of the oil content of oilseeds are very time consuming, with extraction periods of 4 to 8 h. Three different oilseeds—rapeseed, sunflower, and soybean—are extracted by supercritical fluid extraction (SFE), accelerated solvent extraction, microwave-assisted extraction, solid fluid vortex extraction, and Soxtherm, and the results are compared with the result of the German Fat Science Society (DGF) standard method B-I 5 (87). Besides, the extracts are analyzed regarding the content of tocopherols as a parameter for mild extraction conditions and the content of diglycerides and free fatty acids as parameters for the content of more polar lipids. The results of the determination of the oil content under optimal conditions are comparable with the results of the DGF standard method B-I 5 (87). There are no significant differences between the different methods. The content of tocopherols is dependent upon the extraction method and the type of oilseed. The highest content is obtained by SFE. The content of diglycerides and free fatty acids varied according to the oilseed.

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KEY WORDS: Extraction, oil content, oilseeds.

The characteristic feature of oilseeds is the high content of oil, which is normally about 20% or higher. The residue of the oil pressing process is less important and contributes minimally to the value of an oilseed. Oilseeds are an important economical factor in world trade of agricultural products. The knowledge of a seed's oil content is of key interest to the oil milling business because the monetary assessment in the trade of oilseeds is based on this value. The raw material price depends on its oil content. The oil content, as hexane or petroleum extract, is defined as the whole of the substances extracted under the operation conditions specified and expressed as a percentage by mass of the product as received (1). In order to facilitate global trade of oilseeds, different standard methods such as the methods of the German Fat Science Society (DGF) (2), the American Oil Chemists' Society (AOCS) (3), the International Organization for Standardization (ISO) (1), and the Federation of Oils, Seeds and Fats Associations (4) are available. Most of these methods are based on the first automatic solvent extraction apparatus, designed by Franz von Soxhlet in 1939, which involves the extraction of fat from the solid material by repeated washing with an organic solvent, usually hexane or petroleum benzine, followed by gravimetric determination. In the meantime, other different apparatuses that work in a similar manner are available (Goldfisch, Butt, or Bailey-Walker). Some methods, proposed by Folch *et al.* (5), Bligh and Dyer (6), and Sheppard (7) also use solvent combinations made of chloroform/methanol and ethanol/diethyl ether, respectively. These binary solvent systems have been successfully used for total lipid extraction (8,9), because they are more polar organic solvents. In contrast to more nonpolar solvents, like petroleum benzene or hexane, these binary systems are also able to extract more polar components, such as phospholipids (10). The primary disadvantages of all of these methods based on the Soxhlet method are the long time involved and the large volumes of organic solvents that are necessary for a complete extraction.

Other standard methods for the determination of oil content are also available—supercritical fluid extraction (SFE) (AOCS), nuclear magnetic resonance (NMR) (DGF, ISO, AOCS) and near infrared spectroscopy (NIR) (AOCS)—and are discussed by several authors (11–17). NMR and NIR provide very fast and especially nondestructive methods for determination of oil content in oilseeds, but both methods require an intensive calibration by solvent extraction methods (14,15,17).

Besides these nondestructive methods, in the five last years some other new alternative extraction methods have been available. The great advantages of these methods, in contrast to the standard methods based on Soxhlet, are saved time, reduced cost, and increased sample throughput (18–20).

The aim of this work is to compare different methods for determining the oil content of oilseeds using three different oilseeds: rapeseed, sunflower seed, and soybean. The methods used are accelerated solvent extraction (ASE), SFE, microwave-assisted extraction (MAE), solid fluid vortex extraction (fexIKA), Soxtherm 2000, and the DGF standard method B-I (87). The comparison should show the different possibilities and limits of each method. Besides the oil content, the content of tocopherols, as well as the content of diglycerides and free fatty acids, should be determined. Tocopherols are unstable antioxidants, which will be recovered under mild conditions only. Thus, they are good indicators for the extraction conditions showing possible deterioration of the oil as a result of the extraction. The content of free fatty acid and

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diglyceride is used as a parameter for the extraction of more polar compounds and can show the influence on hydrolysis during the extraction procedure.

MATERIALS AND METHODS

Samples. Rapeseed (low erucic acid type), soybean, and sunflower seed (confectionery type), each homogenized and equilibrated from batches of about 20 kg, were used without dehulling or any other pretreatment such as predrying.

For all methods except the DGF standard method, the samples were ground by a Retsch (Haan, Germany) centrifugal mill type ZM100 with a sieve of 0.5 mm size and weight 2 g (sunflower seed and rapeseed) and 4 g (soybean). Each sample was analyzed with each method four times.

DGF standard method B-I (87) (2). Five grams of the seeds were milled with a Dangoumau type laboratory mill (Prolabo, France) using 20 mL of light petroleum benzene (40–60) during milling and 50 mL more to transfer the ground sample into the extraction thimble. Afterward, the extraction was carried out for 4 h in an extraction apparatus according to Twisselmann. After extraction, the solvent was evaporated to dryness and then the extract was dried at 103°C for 2.5 h, cooled for 30 min in a desiccator, and weighed. After further drying at 103°C for 10 min and cooling in the desiccator, the extract was weighed again. This procedure was repeated until the extract obtained a constant weight.

ASE. An automated extraction system (Model ASE 200; Dionex, Idstein, Germany) was used for the extraction. The system consists of a stainless-steel sample cell (11 mL) with equipment for the control of extraction parameters, such as temperature, pressure, extraction time, and volume of solvent. The ground seeds were weighed into the extraction cell, mixed with diatomaceous earth in order to reduce sample compression, and fitted with a cellulose filter and a stainless steel frit at the outlet. The dead volume of the cell was filled with sea sand. The extraction cell was closed and then put into the autosampler. Afterward, the cell was moved automatically into the heatable extraction oven, filled with solvent, and the pressure was adjusted. After the extraction time, the extract was drained off into a collection vial and a new cycle began with filling solvent into the cell, heating, and pressurization. After the last cycle was run, the pipes and the extraction cell were washed with solvent and residues of solvent were purged from the sample into the collection vial by compressed nitrogen. The extract was transferred into a weighed round-bottom flask, and the solvent was evaporated to dryness and dried as described for the DGF method. The conditions were: extraction pressure, 6.67 MPa; extraction temperature, 105°C; heat-up period, 6 min; extraction period (static), 10 min; cycles, 4; extraction solvent, petroleum benzene; solvent rinse, 60% of the cell volume; and nitrogen rinse, 1 MPa for 60 s.

fexIKA 200. The fexIKA 200 is a solid fluid vortex extractor of IKA-Labortechnik (Staufen, Germany). The apparatus consists of a basic vessel in which the extract is collected. This basic vessel is connected to an extraction tube, which is linked to a rod-type cooler. A polytetrafluoroethylene (PTFE) membrane (2 µm) is located (20) between the basic vessel and the extraction tube.

For the extraction, the ground sample material was weighed onto a filter pad, which was placed onto the PTFE membrane. Fifty milliliters of petroleum benzine were added to the basic vessel and 20 mL to the sample in the extraction tube. The apparatus was closed, and the solvent in the basic vessel was brought to a boil with the aid of a heating plate. The vapor from the solvent penetrated the PTFE membrane on which the sample was located. The hot solvent vapor served to heat up and vigorously fluidize the solvent in the extraction tube together with the sample material. At the end of the extraction period, the basic vessel was cooled and the resulting vacuum sucked down the extract from the extraction tube through the membrane in the basic vessel. Afterward, a new cycle started automatically. At the end of the extraction program the extract was transferred into a weighed round-bottom flask, the solvent was evaporated to dryness, and the extract was dried as described for the DGF method (18). Setting parameters for (i) cycle, (ii) $(n-2)$ cycles, and (iii) n cycle were: temperature for heating,140, 140, and 140°C; boiling period, 20, 15, and15 min; temperature for cooling, 50, 50, and 50°C; and filtration period, 2, 2, and 2 min, respectively.

SFE. The apparatus used for SFE consisted of an ISCO SFX 220 (ISCO, Lincoln, NE) with two 100-mL DX syringe pumps for delivery of carbon dioxide and modifier, manual sample handling, and 40°C restrictor heating. The carbon dioxide used was of industrial quality. The ground sample was weighed into the extraction cell, and the remaining void in the cell was filled up with diatomaceous earth to reduce the dead volume of the extraction cell. The collection vessel was filled with about 1 g of glass wool, resulting in a plug of about 2.5 cm high. The vessel was heated at 103°C and weighed. The extraction parameters used were as follows: 517 bar, 100°C, and a total flow volume of carbon dioxide of 120 mL/min. The extract was dried and weighed as described above (21).

Soxtherm 2000. The Soxtherm 2000 is an apparatus of Gerhardt (Bonn, Germany). The ground sample was weighed into an extraction thimble and covered with fat-free cotton wool. The extraction thimble was set into the weighed extraction beaker and approximately 140 mL of petroleum benzine was added. The sample was boiled for 30 min in the solvent under reflux and then the solvent was reduced (5×15 mL) automatically, so that the sample in the extraction beaker was located above the solvent. For a further 80 min, the sample was extracted by the solvent under reflux. Afterward, the residual solvent was removed from the extract by a final heating step, while the extraction beaker was lifted about 1 cm to avoid excessive sample heating. After the termination of the program, the beaker was dried at 103°C as described above (19). The conditions were: boiling time, 30 min; solvent reduction A, 5×15 mL; and extraction time, 80 min; solvent reduction B, 8 min; solvent reduction C, 5 min; solvent reduction interval, 3 min; and solvent reduction phase, 3 s.

MAE. MAE was performed with a Soxwave 100 (Prolabo, France) system. The emission frequency was 2.45 GHz, produced by a magnetron. A program unit allowed power control (max. 300 W) and a choice for the irradiation time. The apparatus was equipped with a reflux column to avoid solvent losses during the extraction. The ground seeds were weighed into an extraction thimble and covered with fat-free cotton wool. The extraction thimble was fixed to a glass rod and inserted into the extraction tube. Fifty milliliters of *tert*-butyl methyl ether was added, and the extraction thimble was lowered into the solvent. Then the MAE was started with P = 30% and t = 60 min. After 20 min, the boiling phase was finished by manually lifting up the extraction thimble while the solvent was heated further. During the subsequent extraction phase, the sample was extracted by the solvent under reflux for another 40 min. At the end of the program, the heating was stopped. The extract was transferred into a weighed round-bottom flask, the solvent was evaporated to dryness, and the extract was dried as described for the DGF method.

Tocopherols. For the determination of tocopherols, the extracts were evaporated to dryness under vacuum purging with nitrogen without further drying at 105°C. About 500 mg of the oil was dissolved in 10 mL heptane. This solution (20 μL) was injected onto a diol phase high-performance liquid chromatography column 25 cm × 4.6 mm i.d. The mobile phase consisted of heptane/*tert*-butyl methyl ether (99:1, vol/vol). The detection was carried out with a fluorescence detector (22). All samples were measured at least three times and quantified using an external standard.

Diglycerides and free fatty acids. Twenty milligrams of the oil was silylated with *N*-methyl-*N*-trimethylsilylheptafluorobutyramide after addition of tricaprin as the internal standard at room temperature for 15 min. Afterward, the solution was diluted with 10 mL heptane and injected on-column in a gas–liquid chromatograph equipped with a short high-temperature capillary column DB1ht (J&W, Folsom, CA), 10 m × 0.32 mm i.d. connected to a 2-m deactivated retention gap and a flame-ionization detector according to Brühl (23).

Data were analyzed by analysis of standard deviation and variance. The Student's t-test, to evaluate the statistical significance for independent and variable interactions, was performed with two-tailed t-tests at P = 0.05. The data was evaluated using a computer program (24).

RESULTS AND DISCUSSION

The oil content of three different oilseeds—rapeseed, soybean and sunflower seed—was determined by six different extractive methods. The methods were optimized for the determination of the oil content regarding the extraction parameters. The optimal conditions were developed step by step, starting from the recommendations of the manufacturer, if available, with the aim to reach the same oil content as reached with the DGF standard method. The influence of different parameters such as extraction time, temperature, or solvent amount of the different methods and the steps of the method development are described elsewhere (18,19,21,25,26).

The results of the determined oil contents are shown in Figure 1. Although the methods use different principles of extraction, there is no significant difference between the results found for the oil content of rapeseed and sunflower seed, respectively (P = 0.05). Only for soybean are there significant differences (P = 0.05) between the methods. One reason might be the much smaller oil content of soybeans compared to rapeseed or sunflower seed. Therefore, the extraction of the oil from soybean seeds is more difficult, making differences between methods more likely.

Statistical analysis shows that for sunflower seed, the variation of the results within one method are greater than the variation of the mean values of the results between the different methods. However, the difference of the variations is not significant (P = 0.05). Obviously for sunflower seed, the characteristic feature method has only a slight influence on the re-

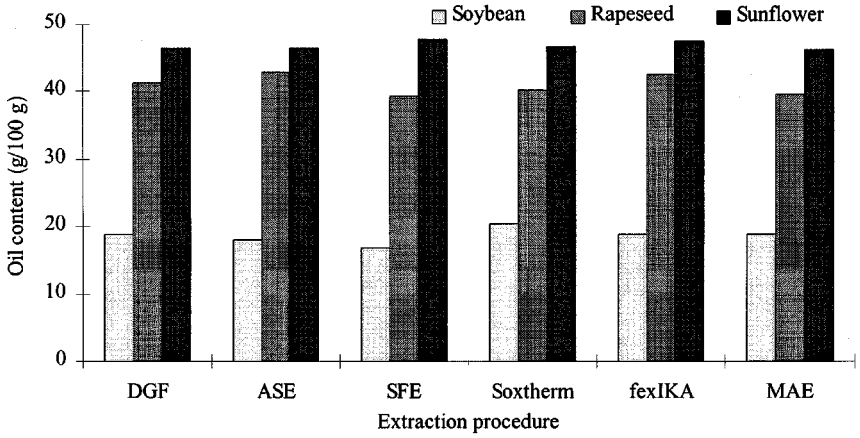

FIG. 1. Oil content of three different oilseeds in respect to the extraction method. DGF, German Fat Science Society standard method B-1 (87); ASE, accelerated solvent extraction; SFE, supercritical fluid extraction; fexIKA, solid fluid vortex extraction; MAE, microwave-assisted extraction.

sults of the oil content; the deviation within each method is greater than the deviation between the methods.

The results for rapeseed and soybean are different from those for sunflower. The coefficient of variance of the results obtained by each method is lower than the coefficient of variance of the mean values of the results of each method. Thus, the influence within each method on the oil content is smaller than the influence of the different extractive methods.

Important distinguishing features between the different methods are the time and the amount of solvent needed for an exhaustive extraction. These points are decisive criteria for the use of such methods in routine analysis. In Table 1, the time and the solvent needs for the different extraction methods are listed. It is obvious that all methods are able to reduce the extraction time considerably in comparison to the DGF standard method. Some methods are able to decrease the amount of solvent needed for the extraction as well. Only Soxtherm used more petroleum benzene, while the other methods needed equal or lesser amounts of solvent than the standard method. Instead of petroleum benzene, the SFE method uses carbon dioxide and the MAE method uses *tert*-butyl methyl ether. The reason for the use of *tert*-butyl methyl ether for extraction with MAE is that it is not possible to bring petroleum benzene to a boil under the conditions used. The requirements for the application of MAE are a sufficient dielectric constant of the sample and/or the solvent, a sufficient boiling point of the solvent, and an appropriate solvent for the exhaustive extraction of the desired compounds or compound classes. Due to the high dielectric constant of water, the water content of the oilseed sample exerts a great influence on heating by microwaves. In the present system, the energy of the microwave apparatus used was limited to 300 W. Because the dielectric constant of petroleum benzene is zero and the water content of the seeds was small, petroleum benzene was not suitable for a sufficient absorption of microwave energy. The dielectric constant of *tert*-butyl methyl ether is about 1.2, so a sufficient absorption of microwave energy was ensured.

Looking at the extraction principles of the different extraction methods explains the differences in their effectiveness. Whereas the standard DGF method uses only a repeated washing of the sample with a solvent under normal conditions (room temperature and atmospheric pressure), the other methods changed the extraction step. All new methods use extraction under elevated temperature of the samples in the solvent, whereas the standard method only uses the solvent refluxed from the condenser. As a consequence of the higher temperature of the solvent, the capacity of the solvent to solubilize analytes increases. For example, the solubility of hydrocarbons can increase several hundredfold when the temperature of the solvent increases from 50 to 150°C (27). Another important point is that the solubility of water in the organic solvent will increase with increasing temperature (28). Under normal conditions the organic solvent may be excluded from water sealed pores which contain analytes. With increasing temperature and/or pressure, the solubility of water in the solvent increases and thus the possibility of the analyte to dissolve in the solvent is enhanced. At higher temperatures the diffusion rates of the analytes increases and hence the extraction rates would be improved.

In detail, the ASE is based on the static extraction of the sample with usual solvents under elevated temperature and pressure for a short time. As a result of elevated temperature and pressure, the solvent remains in the liquid phase. The solubility and mass transfer increase, and the surface equilibria are disrupted (29), which produces enhanced performance for the method. The important difference of the ASE method in contrast to the standard DGF method, fexIKA, Soxtherm, and MAE is the application of high pressure during the extraction. The use of pressure enables the extraction of analytes, which under normal conditions are trapped in pores. Under elevated pressure, the solvent would be forced into the pores, and possibly sealed by water or air to contact the analytes.

During SFE, the sample is extracted under high pressure and temperature using the fact that any substance may exist as liquid at a supercritical fluid state. The properties of the fluid are between a gas and a liquid in this state. As a consequence of lower viscosity and higher solvent diffusion, the mass transfer out of the sample increases, which results in a noticeable reduction of the extraction period. Important to the extent to which the sample is extracted are also adsorptive effects of the matrix, which influence the yield of the extract (30). In most cases carbon dioxide is the extraction solvent of choice for SFE, because of its easy removal from the extract, its low tox-

TABLE 1
Economical Estimates of the Different Methods

Method	DGF	ASE	SFE	fexIKA	Soxtherm	MAE
Time (min)	240	40	40	110	150	60
Solvent (mL)	70	20	120[a]	70	140	50[b]
Possibility of automation	No	Yes	Yes	No	Partly	No
Initial equipment costs	−−	++	++	−	+	+
Technican time/sample (min)	30	15	12	20	15	20
Costs per sample	+	−	−	+	+	+

[a]Carbon dioxide.
[b]*Tert*-butyl methyl ether. −−, very low; −, low; +, high; ++, very high. DGF, German Fat Science Society standard method B-1 (87); ASE, accelerated solvent extraction; SFE, supercritical fluid extraction; fexIKA, solid fluid vortex extraction; MAE, microwave-assisted extraction.

icity and reactivity, and its high purity at relatively low cost (30). Also, environmentally, the disposal of carbon dioxide is no problem in contrast to the classic solvents, petroleum benzene or hexane. Hexane has an acute narcotic and a chronic degenerative effect on the peripheral nervous system.

In the case of the oilseeds the solubility of the triglycerides depends considerably on the pressure and the temperature used for the extraction (30), and, given the appropriate density, most triglycerides are sufficiently soluble in carbon dioxide at temperatures between 60 and 80°C.

The Soxtherm 2000 combines a boiling period and an extraction period. During the boiling period, the sample is placed in the boiling solvent and a great amount of the extractable compounds are solubilized. In a second step, the sample is lifted above the solvent and washed with a condensed solvent, similar to the original Soxhlet method.

During the MAE, the solvent is heated as a result of the absorption of microwave energy by dipoles existing in the solvent or the sample material. The method is divided into a cooking and an extraction step, similar to the Soxtherm. The difference is the support of the extraction by microwaves. As a result of the microwave treatment, the vaporization of water in the sample destroys the cellular system and enables an exhaustive extraction. In contrast to conventional extraction, the heating shows no temperature gradient from the flask to the center of the solution. Therefore an even heating of the solution is ensured.

The fexIKA is based on cyclical succession of vaporization of the solvent, condensation, extraction, vacuum filtration, and vaporization again. The effectiveness of the extraction by fexIKA, in contrast to the standard method, is a result of boiling hot solvent vapor flowing through the solvent/sample mixture. Thus, an intensive vortex of this mixture is obtained, which results in an effective extraction of the oilseeds. The second point which improves the effectiveness of the method is the separation of the solvent from the dispersed sample. During the cooling phase in the basic vessel, a vacuum is formed and the solvent is sucked down from the extraction tube into the basic vessel. This high filtration pressure leads also to an improved result of this extraction method (31).

The order of the methods regarding the extraction times is obvious with the knowledge of their principles. In comparison to the standard method, the Soxtherm reduces the extraction time significantly by using an additional boiling phase in which the sample is exposed directly to the hot solvent. A further reduction of the extraction time is achieved by fexIKA due to vortexing the sample in the hot solvent. A greater exchange between sample and solvent takes place. Using microwaves in combination with a cooking and an extraction step leads to a further reduction of the extraction time. In the case of ASE and SFE, the additional influence of the pressure becomes apparent.

In Table 1, besides the time and solvent needs for extraction by the different methods, some general information about the initial equipment costs, possibility of automation, technician time per sample, and total costs per sample are presented. It is difficult to give exact data because these costs are very different in different countries.

The tocopherol content of the extracted oilseeds is an important indicator for possible alterations of the oil during the extraction procedure. Tocopherols are relatively unstable antioxidants, which stabilize oils as radical scavengers. The antioxidative activity is based on the formation of a "tocopherol–tocopherylquinone redox system" (32). Elevated temperature, occurring during the extraction process, in combination with oxygen attack, leads to formation of radicals in oils containing unsaturated fatty acids. These reactive intermediates are stabilized by tocopherols and lead to a loss of tocopherols during extraction.

Any other heat treatment of the sample, except that needed for extraction, was ruled out during the investigations. The solvent removal was obtained by purging the flask containing the extract with a stream of nitrogen until dryness. No saponification was carried out in order to avoid losses due to alkali treatment. Saponification is only needed for tocopherol esters used in margarines (33). As a result of the gentle treatment of the extracts, it could be assumed that all differences in tocopherol content were due to the extraction procedures. The quantitative amount of tocopherols in the extracts and the individual tocopherol composition were determined.

Figure 2 shows the total tocopherol content of the different extracts. It is obvious that the tocopherol content of the oils is affected by the extraction method. However, the statistical significance at the level of 5% is only valid for results obtained from soybean and rapeseed oils. For sunflower oil, the coefficient of variance is less for the results obtained with one method than the variance between the different methods, but there is no statistical significance. The highest tocopherol content was obtained by the SFE procedure. Extraction by ASE obtained slightly more tocopherols than the standard DGF method for soybean oil. This result is not unexpected because, during the extraction by SFE, the possibility of oxygen to react with unsaturated fatty acids was excluded by the use of carbon dioxide as the extraction solvent. Thus, one important condition for the degradation of tocopherols was avoided. Also, during the extraction by ASE, only a small amount of oxygen was able to come in contact with unsaturated fatty acids, leading to the degradation of tocopherols, because the extraction cell was filled completely with sample material and extraction solvent. Only the amount of oxygen soluble in the extraction solvent under the conditions used during ASE can react with the fatty acids. During the other extraction methods, oxygen has more or less easy access to the fatty acids because the sample is not guarded, but in an open thimble. Especially during the extraction with fexIKA, a vortexing of the sample in an oxygen-containing atmosphere takes place, leading to a strong degradation of tocopherols.

The susceptibility of tocopherols regarding degradation is also influenced by the type of oilseed. While the tocopherols of sunflower seed oil were recovered by all the extraction methods in a similar way, with the DGF method showing the

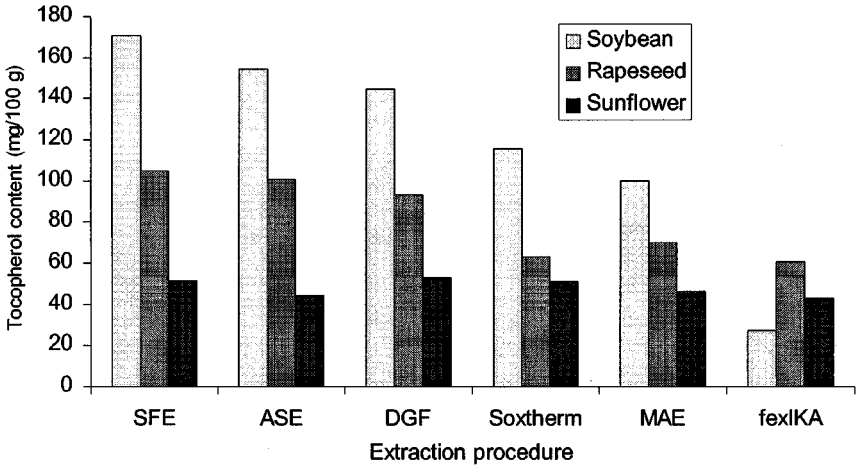

FIG. 2. Total tocopherol content of soybean, rapeseed, and sunflower oil after extraction with different methods. See Figure 1 for abbreviations.

highest content, there were more differences in rapeseed and great variations in soybean oil. These results may be due to the tocopherol composition of the three oils. Sunflower oil is a rich source of α-tocopherol, the tocopherol with the highest *in vivo* activity (34). Rapeseed oil contains predominantly γ-tocopherol with a portion of about 25% α-tocopherol. γ-Tocopherol is the major tocopherol in soybean oil and shows the highest antioxidant activity in oils (35). During the antioxidative reaction of tocopherols, α-tocopherol reacts much faster than the other tocopherols, due to the two ortho-methoxy groups in positions 5 and 7. As a result of this reaction, a radical was formed from α-tocopherol which can start the autoxidation of unsaturated fatty acids (34). Therefore, the formation of hydroperoxides increases with increasing concentration of α-tocopherol. Figure 3 shows the influence of the extraction methods on individual tocopherols of the extracts from different oilseeds. It is obvious that there is hardly any influence of the extraction method on the content of α-tocopherol, whereas the influence on the contents of γ- and δ-tocopherol is significant. α-Tocopherol is the most reactive tocopherol and disappears first from a mixture (36), thus differences among the methods are not apparent. This is different from γ- or δ-tocopherol. In this case, the degradation rate is much smaller so that differences between the different methods are revealed. These results show the different suitability of the extraction methods for further analysis of labile compounds.

The analysis of free fatty acids and diglycerides is suitable to show differences between the extraction methods for their ability to extract the more polar simple lipid classes (37). For an exhaustive extraction of phosphatides, the need of polar solvents is well known (5,8). The extraction of all simple lipids is regarded to proceed uniformly along with the triglycerides. However, there are some variations in the free fatty acid and diglyceride content for the different extracts (Figs. 4 and 5). These differences may be due to incomplete extraction or to a formation of partial glycerides during the extraction procedure. This implicates two mechanisms, which work against each other. MAE and SFE provided the highest amount of free fatty acids, while DGF and ASE rank at the lower level. However, there is not a consistent order of the methods for both lipid classes and all seeds tested. For the

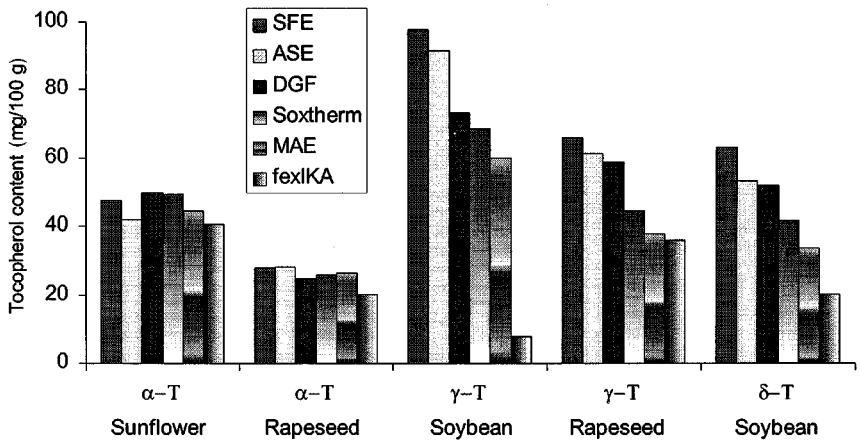

FIG. 3. Content of individual tocopherols in extracted seed oils as a function of the the extraction method. T, tocopherol; see Figure 1 for other abbreviation.

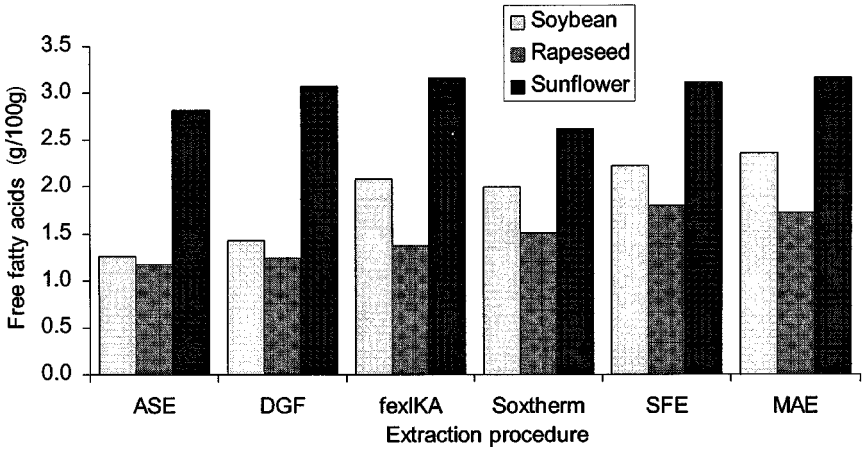

FIG. 4. Content of free fatty acids in soybean, rapeseed, and sunflower oil after extraction with different methods. See Figure 1 for abbreviations.

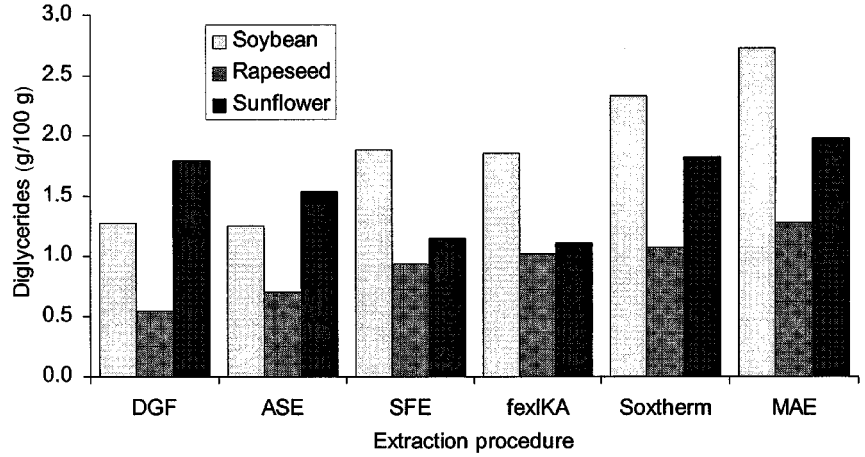

FIG. 5. Content of diglycerides in soybean, rapeseed, and sunflower oil after extraction with different methods. See Figure1 for abbreviations.

content of free fatty acids, the coefficient of variation of the results obtained with one method is lower than the coefficient of variation of the results between different methods. There is no statistical significance to distinguish between the methods. Only the results for the content of diglycerides in soybean oil show a statistical significance to distinguish between the different extraction methods. This may be due to the same reasons for the differences in the results of the oil content.

In summary, it is possible to optimize the six methods to get results for the determination of the oil content for rapeseed and sunflower seed that are not significantly different (P = 0.05). For soybeans there is a significant difference between the methods based on the oil content. In all cases, the triglycerides represent the major lipid class, but the statistical treatment of the data shows that the extracts are not the same.

As shown in Table 2 for rapeseed and sunflower seed, only the parameter tocopherol content of rapeseed has a significant difference between the results of the methods examined. All other differences between the results of these methods are not statistically significant. For soybeans, the differences of the results of most parameters show a statistical significance. Only the results of the free fatty acids are not statistically significant.

However, it is obvious that the variance of the results within each method is lower than the variance between the results of the different methods of almost all parameters of

TABLE 2
Statistical Significance for Differences of the Results Obtained with the Extraction Methods at a Level of 5% Probability for Each Oil and Analysis[a]

	Oil content	Tocopherols	Free fatty acids	Diglycerides
Sunflower	– (–)	– (+)	– (+)	– (–)
Rapeseed	– (+)	+ (+)	– (+)	– (+)
Soybean	+ (+)	+ (+)	– (+)	+ (+)

[a]+, statistically significant difference between the extraction methods; –, no statistical significant difference between the extraction methods; (+), variance of the results within each method lower than the variance of the results between the methods; (–), variance of the results within each method is higher than the variance of the results between the methods.

the three seeds. Only for the oil content and diglycerides of sunflower seed is the variance within each method higher than the variance between the methods.

The results of the statistical analysis show that all methods are suitable for the extraction of sunflower seed, since not only the oil content is comparable but also the composition of the extracted oil is not statistically different. Regarding rapeseed, it should be noted that the content of tocopherols is statistically different between the different methods. In the case of soybean, the oil content and the compostion of the oil is significantly different for the used methods. The definition of the oil content ignores the composition of the oil being extracted, but as shown in the present work this is a critical point, because the composition of the extracts obtained with the different methods is not the same in all cases. So seed traders and processors have to choose the right method for their individual tasks.

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